ImmunoCAP® ECP Conjugate 50

Fluoroenzymeimmunoassay

Directions for Use 52-5239-EN/07

INTENDED USE

ImmunoCAP ECP is an *in vitro* test system for the quantitative measurement of Eosinophil Cationic Protein (ECP) in human serum. ImmunoCAP ECP Conjugate 50 is intended to be used with the instrument Phadia 250 and Phadia 1000.

SUMMARY AND EXPLANATION OF THE TEST

It is known that eosinophilia is associated with a variety of inflammatory disorders including allergic disease. Asthma is such a disease where primed and activated eosinophils are a common finding.

In such diseases eosinophils and their toxic products are seen in tissue specimens from inflammation foci e.g. in asthma (1). The cells and their granule products seem to be major causes of the tissue destruction, e.g. shedding of epithelial cells in the airways (2,3,4). The pathophysiological changes seen in the asthmatic lung leads to hyperreactivity. The grade of inflammation may be identified by the presence of activated eosinophils in the peripheral blood. These cells have the propensity to release their granula constituents during the *in vitro* clotting process (5). This release is enhanced by temperature increase. Determination of released ECP may be one way to monitor conditions involving eosinophil activation.

Inflammatory conditions like acute asthma are treated by anti-inflammatory drugs such as corticosteroids (6). The decline of inflammation, reflected by the released ECP, may be of value in monitoring the effect of therapy.

ECP levels have been found elevated in other clinical conditions connected with activated eosinophils like atopic dermatitis (7), certain infections (8), autoimmune conditions in the joints (9), gut (10) and in some parasitic diseases (11).

PRINCIPLE OF THE PROCEDURE

Anti-ECP, covalently coupled to ImmunoCAP, reacts with the ECP in the patient sample. After washing, enzyme labelled antibodies against ECP are added to form a complex. After incubation, unbound enzyme-anti-ECP is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. To evaluate the test results, the response for the patient samples are transformed to concentrations with the use of a calibration curve.

REAGENTS for Phadia 250 and Phadia 1000

Reagents are packaged in separate units, each purchased separately. The two digit suffix (-01) on the article number may vary between countries. All units are required to perform an assay, though, calibrators are not required for additional assays while the stored curve is valid. The expiration date and storage temperature for each of the units are stated on the outer label.

Note! It is not recommended to pool any reagents.

ImmunoCAP ECP Conjugate 50 (Art No 10-9321-01)

(Fluoroenzymeimmunoassay for 2 x 50 determinations)

ECP Conjugate 50 ß-Galactosidase-anti-ECP (mouse monoclonal antibodies) Approximately 1.5 µg/ml Sodium azide 0.05% Colour coded blue; 2.5 ml	2 vials	Ready for use Store at 2 - 8 °C until expiration date **Do not freeze!**

ImmunoCAP ECP Calibrator Strip (Art No 10-9345-01)

(Reagents for one calibration curve)

ECP Calibrator Strip (human ECP in buffer) Conc. 2; 5; 15; 100 and 200 µg/l Sodium azide 0.05%; 0.2 ml	1 strip The strip contains one calibration curve	Ready for use Store at 2 - 8 °C until expiration date

ImmunoCAP ECP Curve Control Strip (Art No 10-9352-01)

(Reagents for 6 x 1 Curve Controls)

ECP Curve Control Strip (CC1) (human ECP in buffer) Sodium azide, 0.05%; 0.2 ml	1 strip The strip contains 6 CC1	Ready for use Store at 2 - 8 °C until expiration date

ImmunoCAP ECP Anti-ECP (Art No 14-4515-01)

ImmunoCAP Anti-ECP (a-ECP) (mouse monoclonal antibodies) Preservative* <0.003%	Carriers of 16 ImmunoCAP	Ready for use Store at 2 - 8 °C until expiration date

Washing Solution (Art No 10-9202-01)

For information see separate Washing Solution Directions for Use.

REAGENTS for Phadia 250

Development Solution (Art No 10-9441-01/Art No 10-9440-01)

(Reagents for 6 x 200 determinations/Reagents for 6 x 315 determinations)

Development Solution 4-Methylumbelliferyl-ß-D-galactoside 0.01% Preservative* <0.0010%; 11 ml/17 ml	6 bottles	Ready for use Store at 2 - 8 °C until expiration date **Do not freeze!**

Stop Solution (Art No 10-9442-01)

(Reagents for 6 x 185 determinations)

Stop Solution Sodium carbonate 4%, 119 ml	6 bottles	Ready for use Store at 2 - 8 °C until expiration date

REAGENTS for Phadia 1000

Development Solution (Art No 10-9439-01/Art No 10-9314-01)

(Reagents for 6 x 1200 determinations/Reagents for 6 x 2000 determinations)

Development Solution 4-Methylumbelliferyl-ß-D-galactoside 0.01% Preservative* <0.0010%; 65 ml/112 ml	6 bottles	Ready for use Store at 2 - 8 °C until expiration date **Do not freeze!**

Stop Solution (Art No 34-2271-51)

(Reagents for 1200 determinations)

Stop Solution Sodium carbonate 4%; 850 ml	1 bottle	Ready for use Store at 2 - 8 °C until expiration date

***Preservative**: Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1).

⚠ Precautions

- For *in vitro* diagnostic use. Not for internal or external use in humans or animals.
- Do not use reagents beyond their expiration dates.
- This kit contains reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or other local/national guidelines on laboratory safety procedures.
- Reagents containing >0.0015% mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1) may cause sensitisation by skin contact. Avoid contact with skin. Wear suitable gloves. For more information see Safety Data Sheet.
- Reagents that contain sodium azide as a preservative must be handled with care. For more information see Safety Data Sheet.

Handling of ImmunoCAP Carrier

Keep the carrier closed to avoid evaporation of buffer. Do not leave the carrier open for more than 1 day at room temperature, otherwise, discard the first ImmunoCAP.

Indication of instability

Phadia 250 and Phadia 1000 software have built-in acceptance limits for the calibration curve and the curve controls. For more information see Phadia 250 User Manual and Phadia 1000 User Manual.

INSTRUMENTS

Phadia 250 and Phadia 1000 are continuous random access systems that perform all steps of the assay. For further information regarding handling of the systems see Phadia 250 User Manual and Phadia 1000 User Manual.

SPECIMEN COLLECTION AND PREPARATION

Parameters such as blood collection tube, coagulation time and temperature must be kept within specified limits, since they affect the concentration of released ECP in serum samples. The clotting represents the first incubation in the assay, in which the ECP measured is to be reproducibly released from the eosinophils which have been activated by the inflammation.

1. Collect blood by venipuncture using Terumo Venosafe Serum-Gel tubes. It is important that the tube is completely filled. Please, contact Phadia AB if another kind of serum collection tube than Venosafe is to be used.
2. After collection gently invert the tube several times. Do not shake or vortex the tube.
3. Release ECP by clotting for 60 to 120 minutes at room temperature 20-24 °C. The temperature must not vary more than ±1 °C between sampling occasions to give comparable results.
4. Centrifuge at 1000–1300xg for 10 minutes at room temperature.
5. Decant serum into a new tube.
6. Serum samples may be kept at room temperature for shipping purposes. Otherwise store at 2 °C to 8 °C if assayed within five days after collection or at –20 °C if assayed later.

Note! Plasma and haemolyzed serum can not be used.

For information on interfering substances see references (13,14).

Preparation of Samples

No dilution of sample is usually required, however, for determination of values higher than 200 µg ECP/l, samples can be diluted with:

ImmunoCAP IgE/ECP/Tryptase Sample Diluent (10-9360-01)

PROCEDURES

For more information see Phadia 250 User Manual and Phadia 1000 User Manual.

On Board Stability

	Phadia 250	Phadia 1000
Calibrator/ CC -strip	28 days. If >3 days between usage, unload the strip and store in 2-8°C.	
Conjugate	4 days open in 2-8°C if bottles are re-capped every night.	7 days open in 2-8°C.
Development solution	40h open in room temperature. Can be used 5 times during shelf-life and be stored at room temperature for 8h on each occasion. Re-cap bottles every night.	14 days open in 2-8°C.
Stop solution	40h open in room temperature. Can be used 5 times during shelf-life and be stored at room temperature for 8h on each occasion. Re-cap bottles every night.	14 days capped in room temperature.
Wash solution (prepared solution)	7 days in room temperature. Discard every seventh day and perform weekly maintenance according to respective instrument user manual.	
Diluent	7 days in room temperature. Re-cap bottles every night.	N/A
ImmunoCAP Carrier	Until expiration date.	

Parameters of the procedure
Volumes per determination:

Sample	40 µl
Conjugate	50 µl
Development Solution	50 µl
Stop Solution	600 µl

The process time from entering the first sample is 1 hour and 45 minutes.
Incubations are performed at 37°C by Phadia 250 and Phadia 1000.

Procedural steps
For more information see Phadia 250 User Manual and Phadia 1000 User Manual.

Material
Materials provided by Phadia AB:
See under REAGENTS.
Materials required but not provided by Phadia AB:
- Measuring cylinder 1000 ml
- Purified water

Calibration
ImmunoCAP ECP Calibrator Strip is run in duplicate to obtain a full calibration curve. The curve can be stored. Use one ImmunoCAP ECP Curve Control Strip in duplicate to evaluate subsequent assays against the stored curve. For more information see Phadia 250 User Manual and Phadia 1000 User Manual.

Reference material
The ECP Calibrators are calibrated against pure ECP prepared according to Peterson, Jörnvall and Venge (12).

Measuring Range
Undiluted serum 2-200 µg/l.

Quality control
Record keeping for each assay: It is good laboratory practice to record the lot numbers of the components used, the dates when they were first opened and the remaining volumes.
Control Specimens: Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptable ranges.
Controls available from Phadia AB for day to day quality control:

ImmunoCAP ECP Control (10-9269-01)

Proficiency Testing: An external quality assessment program (proficiency testing) is available from Phadia AB for quality assurance purposes (Quality Club):

Quality Club ECP (10-9344-01)

CALCULATION OF RESULTS
All calculations are performed by the Phadia Operator Software. For more information see Phadia 250 User Manual and Phadia 1000 User Manual.

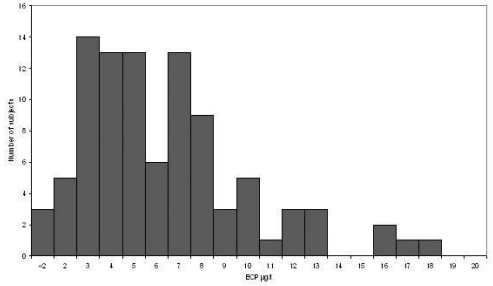
LIMITATIONS OF THE PROCEDURE
A definitive clinical diagnosis should not be based on the results of any single diagnostic method and should only be made by the physician after all clinical and laboratory findings have been evaluated.

EXPECTED VALUES[(a)]
Comparison studies[(a)] between Phadia 100 and Phadia 250 have been performed with 89 samples. Results for samples obtained with Phadia 100 and Phadia 250 show good agreement.
Comparison studies[(a)] between Phadia 100 and Phadia 1000 have been performed with 89 samples. Results for samples obtained with Phadia 100 and Phadia 1000 show good agreement.
Comparison studies[(a)] between Pharmacia CAP System ECP FEIA and Phadia 100 have been performed with 102 samples. Results for samples obtained with Pharmacia CAP System ECP FEIA and Phadia 100 show good agreement.

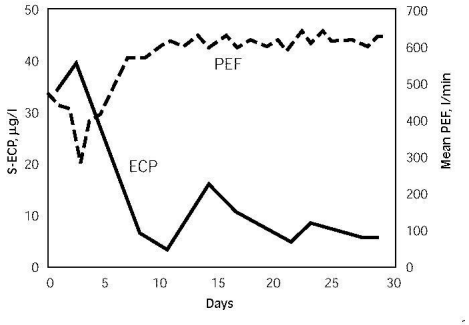
Results obtained for healthy subjects[(a)]
In a study with 95 apparently healthy adults, 44 males and 51 females (age 18-76) the following result was found:

Geometric mean:	5.5 µg/l
90th and 95th percentile:	11.1 and 13.3 µg/l

All samples were collected using Vacutainer SST tubes. The temperature during blood clotting was 20±1°C.
Good laboratory practice dictates that each laboratory establishes its own range of values.



One example of acute exacerbation of asthma[(b)]
During an acute attack of asthma the eosinophils become activated, resulting in elevated levels of ECP in serum. The figure shows ECP in serum and peak expiratory flow (PEF) in a 42 year old patient with an acute asthma. At admittance during an acute attack the patient has high levels of serum ECP. During therapy with corticosteroid the lung function increases to normal and the serum ECP levels decrease to normal, demonstrating that the inflammation is under control.



PERFORMANCE CHARACTERISTICS
Precision[(a)]
The following mean coefficients of variation have been obtained. Each sample has been assayed in 4 replicates on totally 18 different occasions using the same lot of reagents. The results have been obtained with Phadia 1000 using stored calibration curves. They are expected values for both Phadia 250 and Phadia 1000.

Sample level µg/l	Coefficients of variation (%)	
	Within assay	Between assay
2 – 5	6	4
5 – 100	4	4
100 – 200	4	5

Sensitivity[(a)]
The detection limit is <0.5 µg/l.
Specificity[(a)]
Cross reaction was tested with the following results:
EPX <0.1%
Recovery[(a)]
Mean recovery is 87% for both Phadia 250 and Phadia 1000.

WARRANTY
The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Phadia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

SYMBOLS USED

LOT	Batch Code	IVD	*In Vitro* Diagnostic Medical Device
	Biological Risks		Caution
	Temperature Limitation		Contains Sufficient for <n> Tests
	Use By		Consult Instructions for Use
	Manufacturer		

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The ImmunoCAP brand name has replaced the UniCAP brand name.
The Phadia brand name is applied to instrument platforms and related system items.

Notes

[(a)]Studies performed at Phadia AB, Uppsala, Sweden.

[(b)]Study performed at Åre Hospital, Sweden and University Hospital, Uppsala, Sweden.

Patents/Trademarks

The following designations are trademarks belonging to Phadia AB: ImmunoCAP, Phadia, Quality Club.

Venosafe is a trademark of Terumo.

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